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Cytotoxicity Of Some Nano-Pollutants On Allium Cepa Plant

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Abstract

The effects of nano-metals and nano-polyethylene on Allium cepa root cells were studied at different concentrations (1, 5, 10) mg/L after a 5-day treatment period. The genotoxicity study included cellular parameters, including the number and type of chromosomal aberrations, mitotic index, and gene expression of the CDC2 gene. The results of the study showed a significant inhibition of root growth exposed to the nano-suspensions, with the highest inhibition occurring at a concentration of 10 mg/L for all nano-suspensions. The effect was clearly higher on the mitotic index, as the mitotic index decreased with increasing nanosuspension concentration. The greatest effect was at a concentration of 10 mg/L, with an increase in the number and type of chromosomal aberrations, especially sticky chromosomes, which are a sign of high toxicity. All concentrations led to a significant decrease in the number of dividing cells; A concentration of 10 mg/L of nanometals resulted in a reduction in the mitotic index to less than 50% compared to the control group, indicating a sublethal toxic effect. The effect of nanometals and nanopolyethylene was found to significantly inhibit root growth as the concentration increased.Although the cell division index decreased, there was an increase in the expression of the CDC2 gene during this time. This increase could be caused by a number of factors, such as the buildup of cells in the interphase, where DNA damage causes cells to stop in the G2 phase, lowering the mitotic index.

Keywords: *Nanometals, Nanopolyethylene, Allium cepa, CDC2, Chromosomal aberration.*

INTRODUCTION

Despite being in its infancy, nano science is already having an impact on nature and our daily lives. Being an interdisciplinary field, nano science and nanotechnology encompass a wide range of study that calls for collaboration. Nanoparticles, or NPs, are becoming ubiquitous in all facets of our life due to their numerous diverse applications. NPs are found in every industry, including high technology, cosmetics, textiles, medicine, nanomemory and nanorobots (Gamze and Şifa.,2020). But scientists started to consider how these novel compounds would affect the environment and living things. This led to a rise in the number of nanotoxicological research (Al-Subiai et al.,2012; Pfuhler et al.,2013; Azqueta A and Dusinska.,2015; Qassim, et al., 2024).

The unique or improved physical and chemical features of nanomaterials particularly nanoparticles over the last few decades have generated a great deal of interest in them relative to bulk materials. There is no doubting that nanomaterials—physical, chemical, and biosynthesized have many advantageous uses, but they can also have harmful consequences, Mostly because of their small size and increased surface activity, nanomaterials are dangerous. Through cell walls and membranes, they can very easily penetrate and enter biological systems, where they can stay for as long as necessary to carry out their intended functions, They have the ability to cause numerous toxicity consequences, including neurotoxicity brought on by nanomaterials, as well as to delay or prolong the effects that are typically unpredictable (Misra et al., 2012; Navarro et al., 2008; Schwirn et al., 2014; Sun et al., 2007 ; Gao and Jiang, 2017).

According to a number of studies (Adams et al., 2017; Assadian et al., 2018; Katsumiti et al., 2018; Rajput et al., 2018a, c; Servin et al. 2017 ; Mohammed, et al., 2019 ; Qassim, et al., 2021), CuO and ZnO NPs and other NPs are highly toxic to a wide range of organisms, especially for plant growth. Additionally, the weathering of these NPs increases bioaccumulation within the terrestrial food chain. CuO and ZnO NPs are thought to be hazardous either through direct interactions with plant cells and tissues or through the release of ionic forms (Du et al., 2011; Perreault et al., 2014). Further more ,

micro or nano plastic poses a serious cross-border hazard to human health, natural ecosystems, and sustainability because of it is low degradation and unsustainable . manufacturing, use, and disposal (Prata et al.,2019; Silva et al., 2021;AL-Janabi, et al., 2025)

Many studies have been conducted on Allium cepa as a model plant system to evaluate the effects of nano-metals and nano-plastics on the toxic effect on mitosis of plant root cells, thus it is considered a biomarker of cell proliferation under the toxic effect of various pollutants (Maity et al. 2020 ; Ahmed et al.,2018; Sun ZhiQiang et al. 2019 ; Abbas, et al., 2020)

The distribution of chromosomes across two daughter cells after their duplication is the simplest way to characterize the mitotic cell cycle. Reversible phosphorylation of regulatory proteins known as cyclins (CYC) is one of several regulatory mechanisms that tightly regulate progression through the eukaryotic cell cycle.

The activity of a family of cyclin-dependent protein kinases (CDKs) mediates phosphorylation, (Tank and Thaker 2011; John et al. 2001) CDKs are divided into eight classes based on their putative cyclin-binding domains: cyclin-dependent kinases such as CKL and CDKA to CDKG. The CDC2 gene in onions encodes CDKA, the biggest category of plant CDKs (Tank and Thaker., 2011). It is distinguished by the conserved PSTAIRE motif, which binds to CYC (Francis., 2009). In relation to the cell cycle and various plant areas, the cdka genes are expressed more or less constitutively , (Qassim, et al., 2023). CDKAs' mitotic functions are demonstrated by their near proximity to mitotic structures, such as the preprophase band, and their transit contact with chromosomes during the metaphase/anaphase transition (Stals et al. 1997; Boruc et al. 2010) and spindle. Both G1/S and G2/M transitions were shown to involve CDKAs, according to (Hemerly et al.,1995 ; Suhad, et al., 2018).

The study aimed to demonstrate the effect of some nanomaterials such as nano zinc oxide, nano copper oxide, and nano polyethylene at different concentrations on meristematic cell division in A. cepa root cells, as well as to demonstrate nanotoxicity by monitoring mitosis and recording the number and type of chromosomal aberrations during the division stages, in addition to investigating the genotoxicity of nanomaterials by studying the gene expression of the CDC2 gene.

MATERIALS AND METHODS

1-Preparation of Nanosuspension:

To create a stock suspension of ZnO and CuO NPs, 1 gm of the nanometal is directly added to 10 ml of double-distilled water (ddw), then the agglomerates are broken by ultrasonography A at 30% amplitude for 20 minutes. varied amounts of Metal-NPs in diluted solutions, ranging from 1.5 to 10 mg/L. By directly adding (one gm to one liter of ddw) and diluted solution, nano polyethylene(25-35nm) is prepared at a concentration of 1,5,10 mg/l.

2. Plant preparation:

A. cepa plants Bulbs (about 2 cm in diameter) were purchased from an agricultural company in Babylon. Onion bulbs were carefully selected and then planted in glass beakers containing 25 ml of (10-20nm) zinc oxide nanoparticles (ZnO-NPs), copper oxide nanoparticles (Cu-NPs), and polyethylene nanoparticles by 25-35nm with different concenteration (1, 5, 10 mg/L) of suspension solutions. They were left to germinate under laboratory conditions with appropriate lighting and temperature. After three days, the roots reached 2-3 cm in length for the rest of the laboratory tests.

3. Meristematic cell Staining and microscopic test:

0.5 g of basic fuchsin dye was dissolved in 20 ml of 95% ethanol. Distilled water was used to dilute it to 100 ml. The dye solution was filtered using Whatman No. 31.

After cutting the growing roots with a blade to a length of 0.5–1 cm, the roots were placed on a watch glass filled with fuchsin basic dye and placed in a fixing solution consisting of 99% ethanol and

glacial acetic acid. The roots were stored at 4°C in 70% ethanol until examination. The roots were rinsed three times for 1 minute each with distilled water to remove the fixing solution. Drops of 1NHCl were applied to soften the tissue. After three 2-minute washes with distilled water, the roots were dried with paper towels to remove any residual moisture. A heated glass rod was used to repeatedly tap the root tip on the glass slide to soften it. The slides prepared for each exposure medium were examined at 40x magnification using an OPTO EDU light microscope. To score interphase cells, cells in mitosis, and chromosomal abnormalities in dividing cells, at least 1000 A. cepa root meristematic cells were randomly selected from each prepared slide.

4.Mitotic Index study:

Mitotic index (MI) was calculated for root tips of each onion bulb using the following formula (the total number of dividing cells is the cells undergoing prophase, metaphase, anaphase, and telophase stages)

Mitotic Index(%) = number of dividing cells/total number of cells counted × 100.

the phase indices (PI) were calculated:

Phase Index (%) = number of cells in specific mitotic stage/ total number of cells counted × 100

Chromosomal aberrations (CA) were calculated by

Chromosomal aberrations (%) = Number of cells with Chromosomal aberrations/number of dividing cells X 100.

5. Genotoxicity test:
Total RNA Was Extracted From Root Tips Using Direct-Zol™ RNA Miniprep According To The Manufacturer's Instructions Go Taq® 1-Step RT-Qpcr Was Used To Synthesize Cdna From 4µg Of Each RNA Sample.

A Mx3000P (Stratagene, CA, USA) Qpcr System Was Used To Quantitatively Amplify The CDC2 Gene Using The Following Primers:

5'-Ttgtcccaa Gatccctgaag-3' And 5'-Ttatgggtaccccaaacgaa-3'.
After Using The Primers, The Results Were Normalized To Those Of Actin As A Control: 5'-Ggattccagctgcttcca Ttc-3' And 5'-Gcttcccgatgg Tcaagtca-3'.
The Procedure For Amplification Was 95 For 10 Minutes And 37 For 15 Minutes.
Step 95 Of Degeneration For 30 Seconds
RNA Is Transmitted To CDNA At 37 And 95 Degrees. DNA Is Separated For 30 Seconds At 95 Degrees, Primer Connects With Gen For 30 Seconds At 58 Degrees, And The Master Mix Enzyme Begins Constructing And Organizing The DNA At 72 Degrees. DNA Is Now Double-Stripped With Gen, And The Operation Is Repeated Forty-Five Times To Double The DNA. The Average Expression Found In Roots That Received DW Treatment For Three Hours Was Used As A Unit Of Measurement According To (Fouad, A. S., & Hafez, R. M.,2018).

Statistical analysis:

The mean of three replicates ± standard deviation (SD) was used to express the results of each treatment. SPSS version 14 was used to determine the least significant difference (LSD) at P value =0.05 for each parameter under investigation, and Minitab version 10.0 was used to conduct regression analysis.

RESULTS AND DISCUSSION:

Effect of nano materials on the root growth in Allim cepa

These findings demonstrated that, in contrast to the control group, the nanomaterial suspensions reduced the growth of Allium cepa roots. In all of the employed nanosuspensions, root development completely stopped at a concentration of 10 mg/L. At a concentration of 10 mg/L, copper oxide nanoparticles had the biggest impact on onion root growth. This might be because the suspension's concentration caused tissue injury and programmed cell death (Cenkci et al .,2010).
According to our research, ZnO-NPs and CuO-NPs and Nano polyethylene suspension caused substantial toxicity to A. cepa and greatly decreased root growth, which is in line with findings from other studies. In beet, tomato, green pea, maize, cucumber, rye, zucchini, and soybean, among other plants, prior research showed that ZnO-NPs mostly affected plant growth (Bandyopadhyay et al. 2015; García-Gómez et al. 2018; Wang et al. 2018, Qassim, et al., 2019). Lin and Xing (2007) reported that ZnO NPs were also demonstrated to be inhibitive in root elongation; for example, solutions of 10 mg/L nano-ZnO essentially stopped the root elongation of the Allium cepa . Additionally, according to(Chang et al.,2012), the harmful effects of nano copper oxide and the free radicals it produced significantly inhibited root growth.

Additionally, because nano-polyethylene damages membranes, DNA, and proteins and has a toxic effect on root cells, it significantly inhibits root growth. This lowers the mitotc index and interferes with root cell division (Mondal et al .,2022).

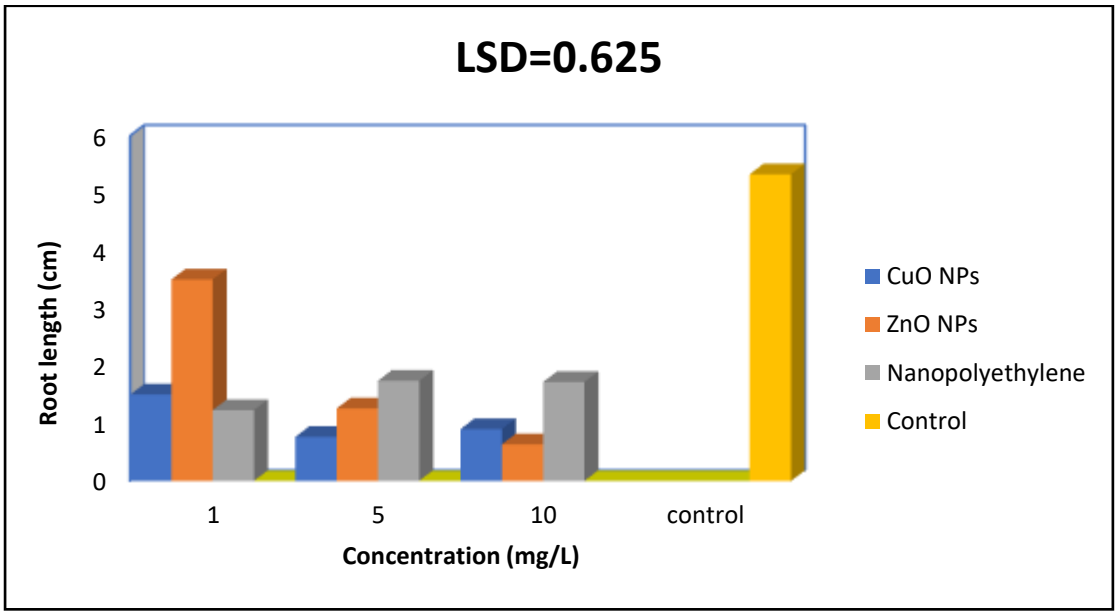

Figure (1): Effect of nanosuspension on the root growth in Allim cepa after 5 days of planting

The effect of nanosuspension on the mitotic index (MI):

The effects of nanosuspension on the mitotic index MI%, and on the division phase index (Prophes, Metaphes, Anaphes, Telophis) were studied as shown in Table 1.The MI index, which calculates the percentage of cells in the mitotic phase of the cell cycle and explains why cell death inhibits it, was used to compare the data in order to assess the harmful impacts of various pollutants on cell division. The results generally show a decrease in the division index of Allium cepa roots after exposure to nano suspension. It was also observed that the division index decreased as the concentration of nanoparticles suspension increased.

The results in Table 1 nano copper oxide led to a decrease in the Mitotic index to 11.66±0.33 at a concentration of 1 mg⁄l compared to the control group, which had a mitotic index of 22±0.57 The mitotic index decreased to 7.33±0.66 at a concentration of 5 mg⁄l compared to the control group, and at a concentration of 10 mg⁄l, which is considered lethal to cells, the mitotic index reached 4.66±0.33

compared to the control group . It was shown that variations in exposure duration and concentration might hinder cell division. Higher concentrations of CuO NPs caused a reduction in mitotic activity over all treatment periods. This work is comparable to earlier research (Jibunor et al., 2014; Nagaonkar et al., 2015). Also The results in Table 1 showed s that Allium cepa roots exposed to nano zinc oxide led to a decrease in the mitotic index to 12±0 at a concentration of 1 mg⁄l compared to the control group, which had a mitotic index of 22±0.57 The mitotic index decreased to 7.66±0.33 at a concentration of 5 mg⁄l compared to the control group, and at a concentration of 10 mg⁄l, which is considered lethal to cells, the mitotic index reached 4.33±0.33 compared to the control group . The findings supported the use of A. cepa plants as a biomarker for the efficient evaluation of metal oxide nanoparticles' cytogenetic effects, particularly those of zinc oxide nanoparticles and Zn2+ ions. According to the experimental results, zinc oxide nanoparticles can enter plant tissues and produce intracellular reactive oxygen species, which can cause oxidative imbalance and have genotoxic and cell division-inhibiting effects. This research aligns with the findings of (Ahmed et al.,2017).

In Table 1 showed the Allium cepa roots exposed to Nano polyethylene also decrease in the mitotic index to 10±0.57at concentration of 1 mg⁄l compared to the control group,which had a mitotic index of 22±0.57 . the mitotic index decreased to 8±0.57 at concentration of 5 mg⁄l compared to the control group ,and at concentration 10 mg⁄l ,which considered lethal to cells the MI reached 7±0 compared to control group . This work supports the findings of Giri and Mukherjee (2022) that these free radicals induce oxidative stress in cells and may also disrupt genes that control division, including the gene cdc2, which is responsible for the division process. As a result, the mitotic index decreases

Table 1: Numbers of dividing cells, division phases, chromosomal aberrations and Mitotic index (MI) for Allium cepa roots cells that exposed to different concentration of nano suspension .

Treatments	Conc.	prophase %	metaphase %	anaphase %	telophase %	Dividing cell %	Chromosomal aberration %	MI%
CuO NPs	1	5.35±0.02	3.22±0.04	1.03±0.006	1.23±0.03	10.71±0.04	11.66±0.33	11.66±0.33
	5	4.10±0.05	0.93±0.01	0.48±0.02	0.28±0	5.74±0.09	38.66±0.33	7.33±0.66
	10	3.34±0.01	0.56±0.01	0.37±0.006	0.27±0.006	4.5±0.03	50.66±0.66	4.66±0.33
ZnO NPs	1	6.20±0.02	2.66±0.20	1.17±0.01	1.28±0.02	11.90±0.02	8.33±0.33	12±0.23
	5	5.10±0.05	0.76±0.06	0.34±0.01	0.42±0.01	6.74±0.01	23±0.57	7.66±0.33
	10	2.60±0.05	0.60±0.02	0.50±0.01	0.39±0.006	4.08±0.04	49.66±0.88	4.33±0.33
Nano PE	1	3.98±0.01	3.12±0.01	0.97±0.01	1.28±0.01	9.28±0.04	45.33±1.45	10±0.57

	5	3.56±0.04	1.88±0.04	0.75±0.01	0.89±0.006	7.14±0.01	55.33±0.66	8±0.57
	10	3.14±0.02	2.04±0.02	0.69±0.006	0.72±0.02	6.62±0.02	77±1	7±0
Control	-	11.28±0.04	6.5±0.05	1.92±0.001	2.38±0.03	22.1±0.05	3±0.74	22±0.57
LSD(P<0.05)		0.122	0.201	0.052	0.066	0.141	0.120	0.113

The effect of nano suspension on the phases index:

The phase index of Allium cepa root samples changed as a result of the nano suspension. as Table 1 demonstrates. The prophase in Allium cepa roots exposed to nano-copper oxide at all concentrations significantly decreased, according to the data, as compared to the control group. At a dosage of 10 mg/L, the prophase dropped to 3.34 ± 0.01 from 11.28 ± 0.04 in the control group, exhibiting the greatest effect. (Figure 2)**.** Also the nano-zinc oxide decreased at all concentration compared to control group the greatest effect was in 10 mg/L which its 2.60±0.05 compared to control group . nanopolyethylene decrease prophase at all concentration the highest effect was in concentration 10 mg/L which its 3.14±0.02. Nano zinc oxide was the highest effect on prophase in all the three nanoparticles. Fig 2.

The samples exposed to nano copper at a concentration of 10 mg/L showed the greatest effect, with the meta phase decreasing comparatively for all nanomaterials and at all doses utilized, by a percentage of 0.56±0.01 in comparison to the control group's 6.5±0.05. When compared to the control group, the greatest effect for nano zinc oxide was at 10 mg/L, with a percentage of 0.60±0.02. For nano zinc oxide and nano copper oxide, the concentrations of 5 mg/L and 10 mg/L seem to have been very comparable. At all doses, nano polyethylene also dramatically decreased metaphase. At a concentration of 10 mg/L, the greatest effect was observed by a percentage of 2.04±0.02. Figure 3 .

For all nanomaterials and concentrations, the anafes phase also dropped comparatively; samples exposed to nanocopper at a dose of 10 mg/L showed the greatest effect, with a percentage of 0.37±0.006 compared to the control group's 1.92±0.001. The concentration of 10 mg/L had the greatest effect on nano zinc oxide, with a percentage of 0.50±0.01 in comparison to the control group. At all doses, nano polyethylene also markedly reduced the anaphase. With a percentage of 0.69±0.006, the greatest effect occurred at a concentration of 10 mg/L. This is seen in Figure 4.

All nanomaterials and concentrations showed a relative decrease in telophase, however samples exposed to nano copper oxide at 10 mg/L showed the greatest effect, with a ratio of 4.5±0.03 to the control group's 2.38±0.03. When compared to the control group, the greatest effect for nano zinc oxide occurred at 10 mg/L, with a ratio of 0.39±0.006. At all doses, nanopolyethylene also dramatically reduced phase separation; at 10 mg/L, the effect was greatest, with a ratio of 0.72±0.02. This is seen in Figure 5.

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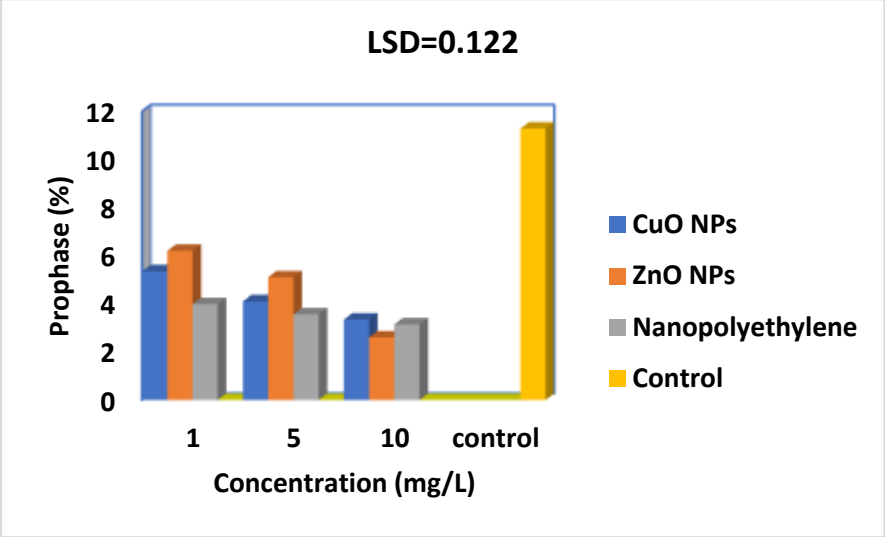


Figure 2: Effect of nanosuspension on the prophase of Allim cepa after 5 days of planting.

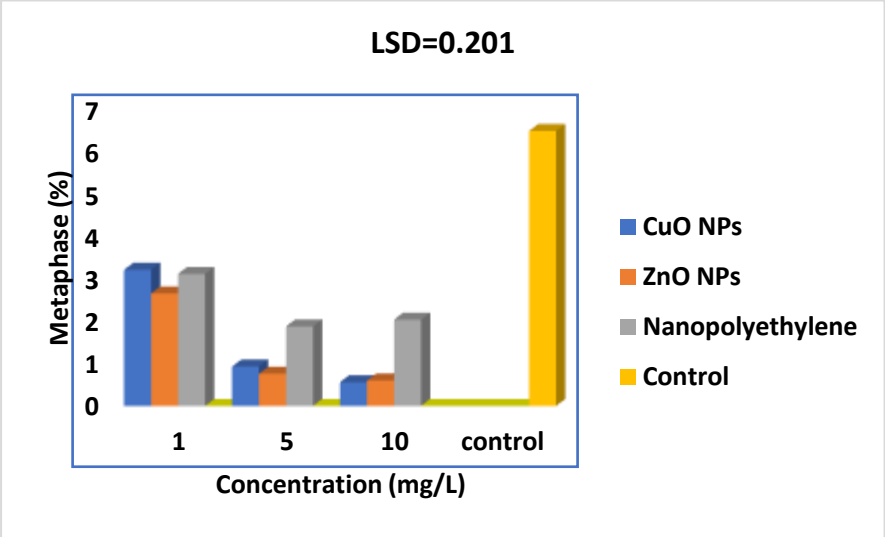


Figure 3: Effect of nanosuspension on the metaphase in Allim cepa after 5 dayes of planting

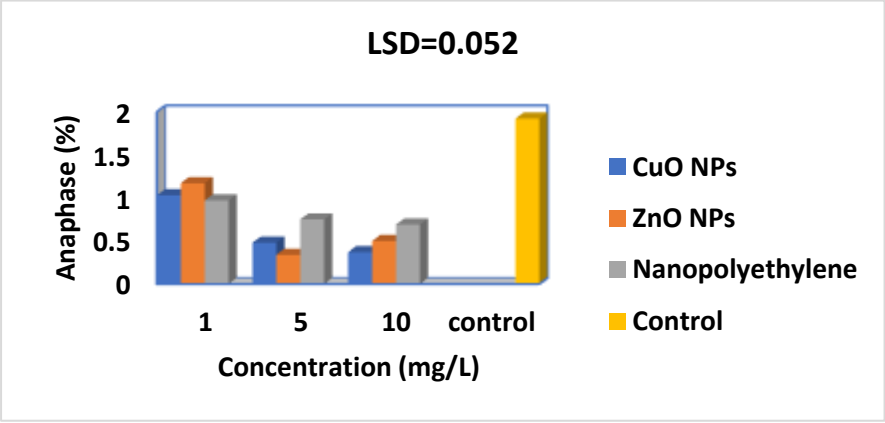


Figure 4: Effect of nano suspension on the anaphase in Allim cepa after 5 days of planting

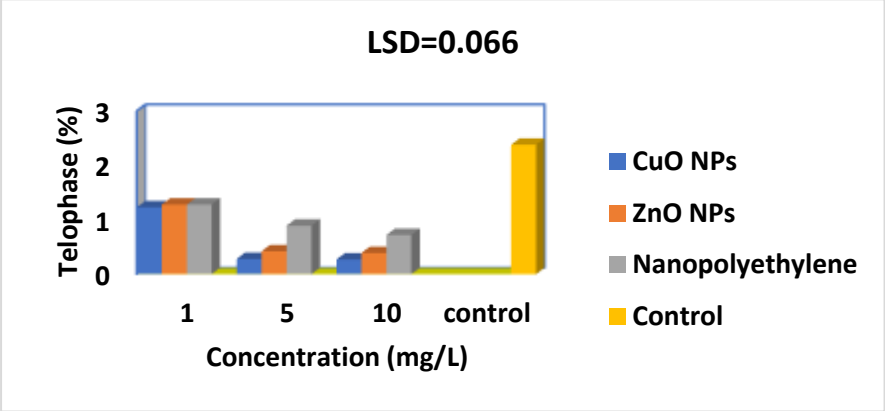


Figure 5: Effect of nanosuspension on the telophase in Allim cepa after 5 days of planting.

Chromosomal aberration:

This study demonstrates that at all doses, nanosuspensions caused some chromosomal aberrations. Additionally, research demonstrates that the percentage of chromosomal aberrations in Allium cepa root cells increased as the concentration of nanosuspensions increased. Numerous studies have discovered various chromosomal aberrations in onion roots exposed to nanosuspensions, and our work is in line with those findings.(Ahmed et al.,2017 ;Jibunor & Elebo.,2024;Gamze & Şifa.,2020)

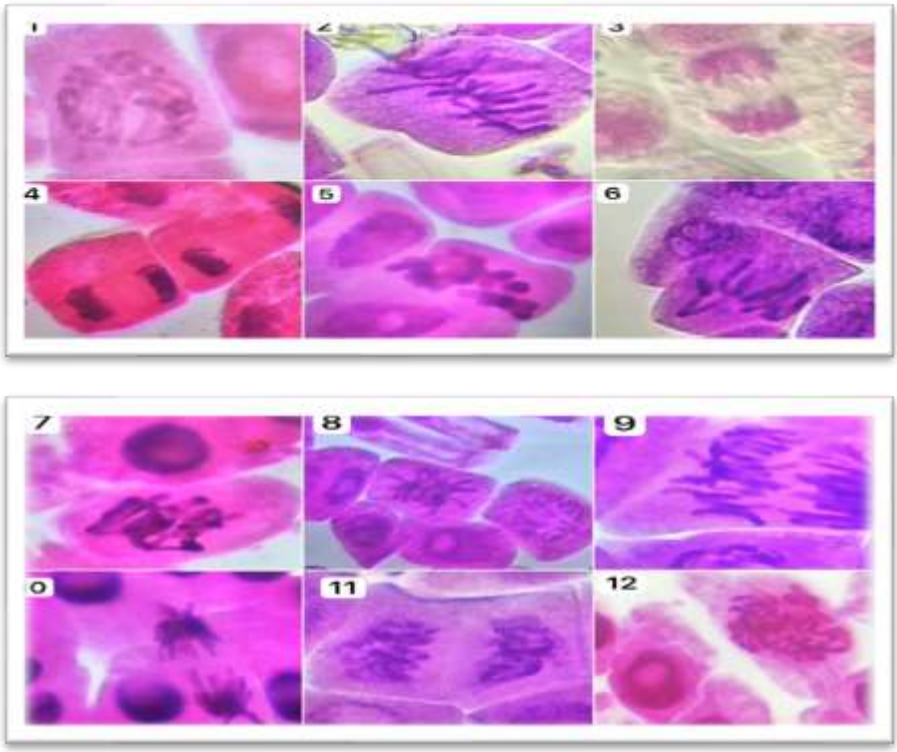
This investigation discovered a variety of chromosomal aberrations caused by exposing Allium cepa roots to varying amounts of suspensions of nanomaterials such as Stickiness, Bridge, Bridge with Laggard, Disturbed, Dispersed, Disoriented, Disorganization chromosomes, as well as Diagonal Anaphase and Micronucleus as shown in table 2 and Fig 6 .

The most common chromosomal defect was the emergence of sticky chromosomes, which can occur for a variety of causes. Sticky chromosomes are a sign of high toxicity of the materials utilized, which typically has an irreversible effect and is likely to cause cell death, according to (Liu et al., 2009). Chromosome or chromatid breaks may be the cause of the formation of chromosomal bridges. The chromatids of the chromosome itself may have broken, and they subsequently properly rejoin from the sticky end.(Amer & Ali.,1974). Additionally, dispersed chromosomes emerged, which are caused by the spindle apparatus's fragility, which results in the chromosomes spreading erratically across the cell (Liu et al., 2009). Other anomalies also emerged, which could be caused by the chromosomes' stickiness or the inversion of their components (El-Ghamery et al., 2009) or by a problem with the spindle apparatus that permits the chromosomes to spread erratically within the cell (Amer and Ali, 1974).

Table 2: Numbers of chromosomal aberration (Stickiness, Bridge, Bridge with Laggard, Disturbed, Dispersed, Disoriented, Disorganization Chromosome, Diagonal Anaphase and Micronucleus) for Allium cepa roots cells that exposed to different concentration of nanosuspension.

Treat.	Con.	Stickiness %	bridge %	bridge with laggard %	disturbed chrom.%	dispersed chrom. %	disoriented chrom. %	chrom. Disorganization %	chrom. Disorganization %	) diagonal %	micronucleus %
Cuo	1	0.39 ±0.01	0.19 ±0.01	0.05 ±0.01	0.05 ±0.01	0.03 ±0.01	0.08± 0.01	0.08±0.01	0.04±0	0±0	0.43±0.01
	5	0.66 ±0.01	0.23 ±0.01	0.17 ±0.01	0.11 ±0.01	0.04 ±0	0.04± 0.01	0.04±0.01	0.09±0.01	0.02 ±0	0.89±0.01

	10	0.52 ±0.0 1	0.27 ±0.0 1	0.10 ±0.0 1	0.07 ±0.0 1	0.02 ±0	0.04± 0	0.04±0	0.23±0. 01	0.05 ±0.0 1	1.02±0 .01
Zno	1	0.41 ±0.0 1	0.21 ±0.0 1	0±0	0.60 ±±0	0.07 ±0.0 1	0.12± 0	0.12±0	0±0	0±0	0.38±0 .02
	5	0.79 ±0.0 1	0.22 ±0.0 1	0.04 ±0	0.05 ±0.0 1	0.02 ±0	0.04± 0	0.04±0	0.04±0	0.04 ±0	0.41±0 .01
	10	0.57 ±0.0 1	0.07 ±0.0 1	0.08 ±0.0 1	0.22 ±0.0 1	0.04 ±0	0.11± 0.01	0.11±0. 01	0.06±0. 01	0.08 ±0	0.69±0 .01
poly	1	0.85 ±0.0 3	0.57 ±0.0 1	0.34 ±0.0 2	0.21 ±0.0 1	0.12 ±0.0 1	0.17± 0.01	0.17±0. 01	0.19±0. 01	0.07 ±0.0 1	0.91±0 .01
	5	0.7± 0.01	0.63 ±0.0 2	0.47 ±0.0 1	0.3± 0.01 1	0.38 ±0.0 1	0.08± 0.01	0.08±0. 011	0.19±0. 01	0.19 ±0.0 1	1.24±0 .01
	10	0.91 ±0.0 6	0.55 ±0.0 1	0.43 ±0.0 2	0.33 ±0.0 0	0.42 ±0.0 1	0.24± 0	0.24±0	0.19±0. 01	0.19 ±0.0 1	1.64±0 .02
Contr ol	-	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
LSD(P <0.05)		0.03 5	0.03 7	0.02 9	0.01 9	0.01 6	0.018	0.018	0.021	0.01 3	0.036



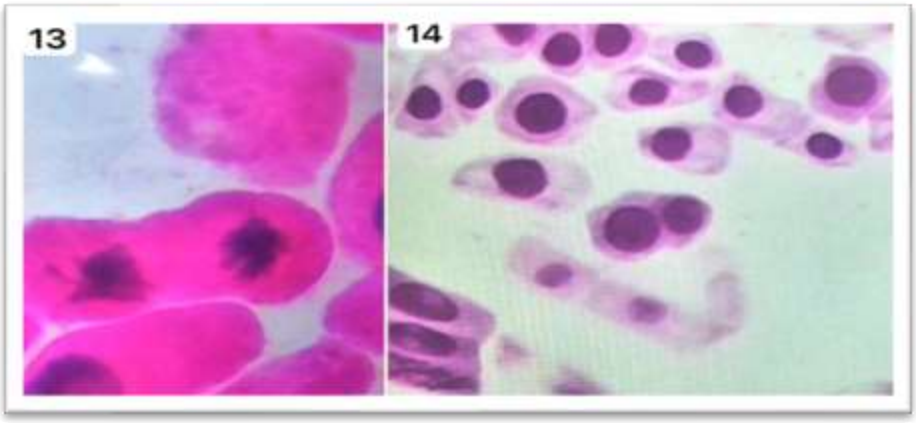


Figure6: Different types of chromosomal aberrations induced by nano suspension in roots tips of Allium cepa (1)normal prophase (2)normal metaphase (3)normal anaphase (4)normal telophase (5)sticky metaphase (6)disoriented metaphase (7)disturbed metaphase (8)dispersed metaphase (9)anaphase chromosome bridge (10)bridge with laggard (11)sticky telophase (12)cell with chromosomal disorganization (13)diagonal (14)micronucleus .

Gene expression of CDC2 gen in Allium cepa root cells:

When evaluating the cytogenotoxicity of environmental threats and extrapolating findings from other animal models, the Allium cepa is the most appropriate in vivo model. (S. Datta et al.,2018 ; W.F. Grant,1978 ; Rodríguez,2015). A gene called CDC2 or CDK (Cyclin-Dependent Kinase) was selected for this investigation. In higher plants, eight different forms of cyclin-dependent kinases (CDKs) have been found to be important cell cycle regulators; CDKA is the most common kind of CDK. The CDKA members are encoded by the CDC2 gene (Tank and Thaker.,2011).

The mitotic cell cycle can be described simply as the duplication of chromosomes followed by their distribution between two daughter cells. Progress through the eukaryotic cell cycle is strictly controlled through several regulatory mechanisms such as reversible phosphorylation of regulatory proteins called cyclins (CYC). Phosphorylation is mediated through the activity of a family of cyclin-dependent protein kinases (CDKs) (John et al., 2001, Tank and Thaker, 2011). Based on the putative cyclin-binding domains, CDKs are classified into eight classes: CDKA to CDKG and cyclin dependent kinases like (CKL). CDKA is the largest group among the plant CDKs, and they are encoded by a cdc2 gene in onion (Tank and Thaker, 2011). It is characterized with conserved PSTAIRE motif responsible for binding to CYC (Francis.,2009). The cdka genes are expressed more or less constitutively both with respect to the cell cycle and different regions of the plant (Hirayama et al., 1991). The mitotic roles of CDKAs are indicated through their transit interaction with chromosomes during metaphase/ anaphase transition (Stals et al., 1997) and by localization closely to mitotic structures including preprophase band.

In this study, the gene expression of CDC2 was measured in onion roots exposed to two different concentrations of the three chemicals, the highest and lowest concentrations of nano suspension which were selected .The three tested materials significantly ($P < 0.05$) enhanced the expression of CDC2 gene compared with the control group, especially at the highest dose of 10 mg/L, according to the data shown in Table (3) and Figure (7). ZnO nanoparticles showed the strongest effect in increasing the expression of CDC2 gene at the same time, followed by CuO nanoparticles and Nanopolyethylene. The expression of CDC2 gene increased significantly ($P < 0.05$) for all tested materials when the concentration increased from 1 mg/L to 10 mg/L. According to the statistical analysis, the observed differences in gene expression between the treated groups and the control group were statistically significant.

Table3 : Gene expression (fold change) in Allium cepa roots cells after exposed to nano suspension

Groups	Concentration (mg/L)	
	1	10
CuO NPs	2.27±0.05	4.39±0.32
ZnO NPs	3.64±0.26	9.17±0.11
Nano polyethylene	1.37±0.04	3.30±0.59
Control	1±0	
LSD (P<0.05)	0.767	

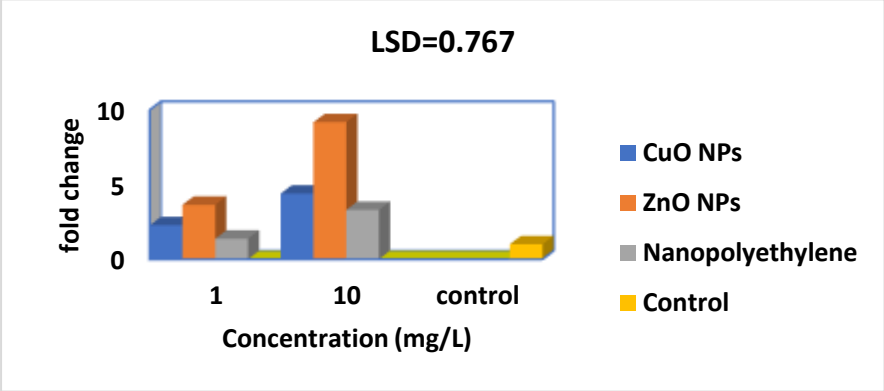


Figure7 : Gene expression (fold change) in Allium cepa roots cells after exposed to nano suspension .

The cell cycle of eukaryotic cells is regulated by many checkpoints. Cell cycle checkpoints measure the cell's growth, guarantee precise chromosome replication, and guarantee chromosome integrity prior to mitosis. The proper segregation during mitosis is then initiated by the metaphase checkpoint via the mitotic spindle. The cell cycle checkpoint requires the regulatory protein cyclins to undergo reversible phosphorylation. This phosphorylation is mediated by the CDK family of activities (Fouad and Hafez., 2018).

According to Ding et al. (2020), CDKs are regulatory proteins that control transcription and limit cell division in response to undesirable circumstances. Eight categories of CDK are distinguished by their alleged cyclin-binding domains.

The CDC2 gene in V. Faba encodes one of them, CDCA (Binarova et al. 1998). CDC2 expression is upregulated prior to cell division (Hemerly et al. 1993) .

These findings demonstrated that, in comparison to the control group, exposure of onion roots to varying doses of nanopollutants resulted in a considerable increase in CDC2 gene expression. There are a number of explanations for these findings, including: Cellular adaptive response: Upon exposure to stress from nanopollutants, cells may initiate compensatory mechanisms to preserve their division capacity, resulting in the activation of CDC2 gene expression to promote cell division and mitigate damage (Hossain and Komatsu, 2015). Oxidative stress: Nanoparticles induce oxidative stress, resulting in the activation of numerous genes linked to the defense response, including those regulating the cell cycle (Hossain and Komatsu, 2015). Variation in cell response: Acute damage from pollutants can sometimes lead cells to fail to complete the division cycle after displaying an initial increase in gene

expression as part of a quick response to harm. (Hossain and Komatsu, 2015). Decreased mitotic index despite increased gene expression. Despite the increased gene expression of CDC2, the mitotic index (MI) decreased significantly with increasing concentration of nanopollutants. This discrepancy can be explained by several reasons: Accumulation of cells in interphase: Increased gene expression does not necessarily mean increased actual mitotic activity, as cells may arrest in the G2 phase due to DNA damage, leading to a decreased overall mitotic rate(Al-Gubory ., 2014). Mitotic depression may be due to decrease in cyclin dependent kinases (CDKs) activity (Zhao et al. 2014), accumulation of cells at G1 phase inhibiting DNA synthesis or arresting the cell in G2, hindering the cell to enter M phase (Mahfouz and Rayan 2017) Cytotoxic effect of nanopollutants: Several studies have shown that exposure to nanoparticles leads to DNA damage, disruption of microtubules, and changes in chromosome structure, preventing cells from entering actual mitotic phase (Nair., 2016).Exposure to NPs potentially leads to toxic side effects such as enhanced ROS generation, disruption of redox homeostasis, lipid peroxidation, impaired mitochondrial function, and membrane damage Activation of apoptosis mechanisms: Some cells may resort to apoptosis in response to severe stress caused by pollutants, leading to a decrease in the total number of dividing cells (Sharma and Chaudhary ., 2024). General interpretation of the relationship between increased gene expression and decreased mitotic rate : The interaction between CDC2 gene expression and cell division can be summarized as follows: When exposed to low concentrations of nanopollutants, compensatory mechanisms may be activated, leading to increased gene expression with limited effect on mitosis. When exposed to high concentrations, DNA damage occurs, causing cells to inhibit entry into mitotic phase despite increased gene expression, leading to decreased mitotic rate. The effects vary depending on the type of nanoparticle, concentration, and duration of exposure, meaning that some particles may be more toxic than others.

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